**156-Plat**
**Structural Coupling of the EF Hand and C-Terminal GTPase Domains in the Mitochondrial Protein Miro**
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The outer mitochondrial membrane protein Miro is a highly conserved calcium-binding GTPase that is at the regulatory nexus of several processes, including mitochondrial transport and autophagy. Miro attaches mitochondria to the microtubule-based motor protein kinesin-1 and acts as a calcium-dependent switch for mitochondrial movement. Phosphorylation of Miro by Pink1 kinase and its subsequent Parkin-mediated degradation leads to mitophagy of damaged mitochondria. Relatively little is known about the molecular underpinnings of these processes and a structural understanding of the relevant protein machinery is lacking. Here we present crystal structures comprising the tandem EF hand and C-terminal GTPase (cGTPase) domains of Drosophila Miro. The structures reveal two previously unidentified "hidden" EF hands, each paired with a canonical EF hand. Each EF hand pair is bound to a helix that structurally mimics an EF hand ligand. A key nucleotide-sensing element and a Pink1 phosphorylation site both lie within an extensive EF hand/cGTPase interface and may have implications for Pink1-mediated recruitment of Parkin to the mitochondrial surface. Our results suggest structural mechanisms for calcium, nucleotide, and phosphorylation-dependent regulation of mitochondrial function by Miro.

**157-Plat**
**Interaction of the BAK Homodimer with the Membrane**
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Programmed cell death, or apoptosis is an essential biological process in embryogenesis or in the maintenance of homeostasis in higher eukaryotes. In the mitochondrial apoptotic pathways, the pro-apoptotic Bcl-2 family proteins BAK and BAX, upon activation by death signals, are believed to form large oligomeric pores *via* homodimer formation in the mitochondrial outer membrane. Through these pores of an unknown structure, many apoptotic factors are released from the intermembrane space into the cytoplasm, where they initiate the cascade of events that lead to cell death. We have determined the topographic locations of the residues in the helices α4, α5 and α6 of BAK in the BAK oligomeric pore using the site-directed spin labeling method and/or the IASD (4-acetamido-4′-((iodoacetyl)amino)stilbene-2,2′disulfonic acid) labeling approach. Accessibility parameters of oxygen and NiEDDA to residues in helix α5 showed that the helix is exposed to the membrane on one side, with the opposite side in tertiary contact. The IASD labeling pattern in helix α5 also revealed the same. These results were consistent with the predictions by the BAX 'BH3-in-groove homodimer', a recently reported X-ray crystallographic structure of a dimer of a truncated BAX consisting of helices α2-α5. The membrane immersion-depths of selected residues in helices α4 and α5 indicated that the adsorption of the BAK 'BH3-in-groove homodimer' to the membrane surface is mediated by the hydrophobic surface of the homodimer. The helix α6 was also adsorbed to the membrane surface, with its N-terminus deeper than the C-terminus. In summary, the data suggest that BAK proteins form a lipidic pore, unlike the channel forming domains of certain bacterial toxins such as diphtheria toxin or colicin molecules. The results provide further insights into the mechanism of formation of the mitochondrial apoptotic pores by BAX or BAK.

**158-Plat**
**Fission Promotes Respiration and ROS Production in Individual Mitochondria**
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The balance between fission and fusion essentially participates in mitochondrial biogenesis, transportation and maturation. Whether endogenous fission and fusion processes regulate mitochondrial respiration and ROS production in the heart is not well studied. Cardiac myocytes are excitable cells that featured by dynamic intracellular calcium regulations. Increased intracellular calcium is known to activate dynamin-like protein 1 (DLP1), a key regulator of mitochondrial fission. In this study, we used adenovirus mediated gene transfer of a mitochondrial targeted superoxide indicator and confocal microscopy to investigate the role of fission in mitochondrial respiration and ROS production regulation. We monitored the mitochondrial superoxide flashes (SOFs), which are quantal events of superoxide generation coupled with single mitochondrial respiration, in cultured cardiomyocytes. Acute application of Mdivi-1 (50 μM), a DLP inhibitor suppressed resting SOF activity in rat adult cardiomyocytes and H9C2 cardiac myoblasts. Furthermore, metabolic substrates (20 mM Pyruvate or 20 mM Glucose)-induced SOF events were blocked by Mdivi-1, indicating an essential role of fission in maintaining normal mitochondrial function. Acute stimulation of fission by KCl (50 mM), which induced calcium transients, dramatically stimulated the SOF (SOF frequency from $0.97 \pm 0.36$ to $2.46 \pm 0.34$ per 1000 μm2 per 100 s) in H9C2 cells. The effect of KCl is blocked by Mdivi-1. Interestingly, long-term incubation of high glucose (35 mM for 48 hr), which has been shown to induce mitochondrial fragmentation, failed to stimulate SOF activity in H9C2 cells. These results reveal an essential role of mitochondrial fission in regulating physiological functions of individual mitochondria and also highlight that chronically disturbing fission/fusion dynamics may have detrimental effects.

**159-Plat**
**The Correlation Between UCP Expression UND Cellular Metabolism**
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Mitochondrial anion carriers transport components of cellular metabolic pathways across the inner mitochondrial membrane. In contrast, the members of the uncoupling protein subfamily (UCP1-UCP5) were shown to transport protons, that is, in case of UCP1, a molecular basis of non-shivering thermogenesis. The exact biological functions of the other UCPs still remain elusive. However, there is increasing evidence that the UCPs' function may be linked to the cell metabolism. In our previous work 1,2,3, we have shown that the tissue distribution of particular UCPs are strikingly different. Here, we hypothesized that their expression is tightly connected to a certain type of cell metabolism. To prove this hypothesis, we employ mouse embryonic stem cells (mESC) which only express UCP2. After initiation of the neuronal differentiation, the UCP2 expression drops abruptly. In contrast, the expression of UCP4 starts with the beginning of neuronal differentiation and lasts throughout neuronal development in embryonic mouse tissue as the expression of other neuronal markers simultaneously takes place. Notably, UCP4 is not present in neuroblastoma cells, which instead prominently express UCP2. To elucidate the role of UCP2 and UCP4 we analyse the regulation of UCP2 expression in cultivated neuroblastoma cells and UCP4 in primary neuronal culture under different growing conditions. The results support our hypothesis that both proteins are a fixed part of the respective cell metabolism.
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